Broken Eggs Influence on Fertilization Capacity and Viability of Eggs, Turbidity and pH of Ovarian Fluid and Fertilization Water in the Endangered Caspian Brown Trout, *Salmo Trutta Caspius*

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Abstract

To identify a simple tool for quick evaluation of quality of endangered Caspian brown trout, Salmo trutta caspius eggs, the changes of pH (both in ovarian fluid and fertilization water), turbidity (both in ovarian fluid and fertilization water), fertilization and eyeing rates were investigated in ovarian fluid samples containing perfect eggs as well as different concentrations of broken eggs. The pH of ovarian fluid and water as well as fertilization and eyeing rates decreased significantly ($P<0.05$) with increasing of broken eggs in ovarian fluid. In contrast, the turbidity of ovarian fluid and water as well as mortality rate of eggs increased significantly ($P<0.05$) with increasing of broken eggs in ovarian fluid. Also, significant correlations ($P<0.01$) were found between measured parameters as follow: pH of ovarian fluid vs. pH of water and fertilization and eyeing rates; turbidity of ovarian fluid vs. turbidity of water; turbidity of ovarian fluid and water vs. fertilization and eyeing rates. Our results conclude that pH and turbidity of ovarian fluid and water effectively influence on efficiency of artificial propagation. Therefore, these could be used as two simple tools for quick evaluation of quality of Caspian brown trout eggs during artificial reproduction in the hatchery.

Keywords: Broken eggs, pH, Turbidity, Caspian brown trout

1. Introduction

The Caspian brown trout, *Salmo trutta caspius*, is a vulnerable anadromous fish considered to biological conservation program in southern part of Caspian Sea (Kiabi et al., 1999; Niksirat & Abdoli, 2009). During last decades, the artificial seed production has been a good strategy for replenishing of Caspian brown trout stocks in sea. As well as, numerous studies have been conducted on the various aspects of reproduction in order to elevation of the efficiency of artificial fertilization. For example, the cryopreservation of semen (Sarvi et al., 2005), seasonal aspects of semen quality (Hajirezaee et al., 2010a,b,c) and in *vitro* storage of ova (Niksirat et al., 2007).

After ovulation (i.e. egg release from the follicles), the eggs are plunged in ovarian fluid, a liquid media composed of organic and inorganic components which preserve eggs till spawning or stripping (Bayunova et al., 2003). At the time of fertilization for salmonidae, the technicians of propagation prefer to elimination of ovarian fluid from eggs since some samples of ovarian fluid inhibit the activity of spermatozoa. It is likely that secreted components of damaged eggs (mostly yolk) and declining of pH establish such effect (Wilcox et al., 1984; Billard, 1992), although the mechanism of this event is not fully understood.

In this study, we examined the effects of broken eggs into ovarian fluid on fertilization rate and viability of perfect eggs of Caspian brown trout. As well as, the turbidity and pH of ovarian fluid were measured as two indicators of egg disintegration in fish. Such study can accumulate more information on fertilization success and could be useful for promotion of artificial propagation about this endangered fish.

2. Materials and Methods

The experiment was carried out at the Kalardasht Salmonids Reproduction Center (KSRC), Iran. The brooders were captured from spawning regions during spawning season (November to December). In KSRC, the brooders were checked up biweekly in order to detection of mature fish. A number of Five and four mature females (females: TL= 28.2 ± 1.3 cm, Weight = 236.4 ± 51.4g) and males (males: TL= 30 ± 4.3 cm, Weight = 306.5 ± 139.1 g) were selected respectively and transferred to hatchery for collection of genital materials. The ovarian fluid containing eggs as well as semen samples were collected by handstripping i.e. massage from the anterior portion of the abdomen towards the genital papilla. Afterward, the eggs were separated from ovarian fluid by a net with tiny meshes. Then, ovarian fluid samples were mixed and distributed in 6 Petri dishes (a quantity of 20 ml ovarian fluid for each Petri dish in 3 repetition) containing 0, 1, 3, 5, 10, 20 eggs broken by scalpel blade respectively and 100 perfect eggs. After mixing, the pH and turbidity (in fact, the Total Suspended Solid (TSS) was measured as a good indicator of turbidity by a portable set (Eutech, PCD 650)) were measured in two stages as follow: a) pH and turbidity of ovarian fluid after mixing with broken eggs. b) pH and turbidity of added water to ovarian fluid containing perfect and broken eggs after fertilization with spermatozoa. The ovarian fluid and water pH were determined with an electrode using a SM102 pH Meter. For fertilization, 1 ml of pooled semen of four males with good quality (with assessment of sperm density and percentage and duration of motility according to Hajirezaee et al., 2010a) was mixed with ovarian fluid containing perfect and broken eggs for each Petri dish and then, the eggs of each Petri dish was incubated in separate incubator. After approximately 8 days, the fertilization rate was determined according to Bromage and Cumaranatunga (1998) where the 50 eggs were sampled randomly from each incubator and then fixed in formalin solution (5 mL formalin (40%) + 45 mL water). then, eggs were investigated under a Stereomicroscope (Meiji EMZ-1). The eggs with visible nervous cord in the back of larval body were considered as fertilized eggs and others without such state were considered as unfertilized eggs. During incubation period, the eyeing and hatching rates determined by an objective method i.e. with counting of eyeing and non-eyeing eggs as well as hatched and non-hatched individuals.

2.1 Statistical analysis

The SPSS software was used for data analysis. The values of pH and turbidity were normal according to Kolmogorov Smirnov test but because of percentage data (fertilization and eyeing rate) did not have a normal distribution, proportional data were converted by angular transformation ($\arcsin\sqrt{p}$). One-way analysis of variance (ANOVA) was employed to compare the means of pH and turbidity for ovarian fluid and water. Where significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which means were different. Also, all correlations were tested using the bivariate correlation coefficients of Pearson.

3. Results

The pH of ovarian fluid and water (Figure 1, $P<0.05$) as well as fertilization, eyeing and hatching rates (Figure 2, $P<0.05$) significantly decreased with increasing of broken eggs in ovarian fluid. In this regard, the percentage of fertilization and eyeing together and hatching strongly decreased to zero for Petri dishes with more than five and three broken eggs respectively. In contrast, the turbidity of ovarian fluid and water (Figure3, $P<0.05$) significantly increased with increasing of broken eggs in ovarian fluid. Significant correlations were found between measured parameters in this study. These correlations were as follow: a) positive correlations: pH of ovarian fluid *vs.* pH of water and fertilization and eyeing rates (Table1, $P<0.01$); turbidity of ovarian fluid *vs.* turbidity of water (Table1, $P<0.01$); b) negative correlations: turbidity of ovarian fluid and water *vs.* fertilization and eyeing rates (Table1, $P<0.01$).

4. Discussion

Several studies have been focused on detection of indicators of egg quality in salmonidae since this problem is an important concern for fish culturists in terms of economy and efficient management of fry production. The quality of fish eggs can be affected by numerous factors, including genetics, nutrition, stress, health status, water temperature, handling and time after ovulation (for review, see Kjørsvik et al., 1990; Bromage, 1995; Izquierdo et al., 2001; Schreck et al., 2001). Such multifactorial constitution of egg quality makes it difficult to control. Therefore, the access to a simple tool for evaluation of egg quality would be useful in terms of time and cost saving. Until now, a numerous of egg characteristics have been used for evaluation of egg quality. These characteristics are: morphological state of eggs (Bromage, 1995), estimation of the percentage of overripe eggs (Barnes et al., 2000), determination of the relative weight increase during 30 min of hardening after fertilization (Lahnsteiner & Patzner, 2002) and measurement of biochemical parameters of ovarian fluid and eggs (Lahnsteiner et al., 1999; Lahnsteiner, 2000). All mentioned procedures are time and cost consuming and require additional skilled laborand and equipment. Thus, the establishment of a simple and quick method is necessary for determination of egg quality of fish.

As quick tests, two previous studies have used from ovarian fluid pH (Dietrich et al., 2007) and water turbidity created by broken eggs after fertilization (Wojtczak et al., 2004) for evaluation of quality of rainbow trout eggs.

In this study, we examined these last methods for endangered Caspian brown trout. The suitability of these tests for this endangered species can prevent from exceeding waste of eggs created by mixing of poor and high quality eggs.

In Caspian brown trout, the pH of ovarian fluid decrease with increasing of broken eggs. As well as, the turbidity of ovarian fluid increased with increasing of broken eggs. These results are in agreement with previous reports on rainbow trout, *Oncorhynchus mykiss* (Wojtczak et al., 2004; Dietrich et al., 2007). The addition of broken eggs and subsequent decreasing of ovarian fluid pH in this study could be a direct evidence for this claim that content of eggs causes a decrease of pH of the fish ovarian fluid. Such declining in pH may be due to the slightly acid pH of egg content as well as relatively weak buffering capacity of the ovarian fluid. Therefore, we can conclude that when low pH of rainbow trout ovarian fluid is observed it is likely that such samples are contaminated with content of the broken eggs.

In salmonids, it was demonstrated that the ovarian fluids with low pH can not activate the motility of spermatozoa at the time of fertilization (Wojtczak et al., in press) and thus, the fertilization rate decreases dramatically. Such event could be possible for Caspian brown trout since there was a positive correlation between pH of ovarian fluid and fertilization rate.

In the present study, the turbidity of water and ovarian fluid increased with increasing of the number of broken eggs. Such turbidity could be due to the releasing of egg content (mostly yolk materials) into the ovarian fluid. On the other hand, a negative relationship was recorded between fertilization rate and turbidity (both in ovarian fluid and water). It is well known that egg content originating from ruptured egg coagulates upon contact with water and causes significant reduction of fertilization rate (Carl, 1941; Wilcox et al., 1984). It is likely that particles originating from broken eggs reduce the fertilization capacity through immobilization or agglutination of spermatozoa or obstruction of the micropyle. One way for minimizing of these effects is using of fertilization solutions where these solutions prevent from coagulation of particles on eggs.

In this study, it was observed that with increasing of ovarian fluid turbidity and decreasing of ovarian fluid pH, the mortality rate increased during incubation of eggs. Therefore, in addition to incubation conditions, it is likely that the decrease of ovarian fluid pH in company with increasing of ovarian fluid turbidity inopportunely decrease the viability of eggs during incubation.

5. Conclusion

The results of present study showed that pH and turbidity of ovarian fluid and fertilization water significantly influence on efficiency of artificial propagation. Therefore, these could be used as two simple tools for quick evaluation of egg quality in Caspian brown trout. As well as, using of fertilization solution is advised for Caspian brown trout since salt solutions reduce the adverse effects of scattered content of broken eggs into ovarian fluid.

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Table 1. Correlations between measured parameters from Caspian brown trout (including: ovarian fluid pH, pH of fertilization water, ovarian fluid turbidity, turbidity of fertilization water, fertilization and eyeing rates). Statistically significant relationships are indicated as follows: ** $P < 0.01$, Data: bivariate coefficient.

	Water turbidity	**Ovarian fluid turbidity**	**pH of water**	**pH of ovarian fluid**	**Fertilization rate (%)**	**Eyeing rate (%)**	**Hatching rate (%)**
Water turbidity	°	0.98	-0.94	-0.98	-0.90	-0.91	-0.84
Ovarian fluid turbidity	**	°	-0.91	-0.98	-0.87	-0.87	-0.77
pH of water	**	**	°	0.94	0.91	0.92	0.88
pH of ovarian fluid	**	**	**	°	0.92	0.92	0.84
Fertilization (%) rate	**	**	**	**	°	0.99	0.89
Eyeing rate (%)	**	**	**	**	**	°	0.90
Hatching rate (%)	**	**	**	**	**	**	°

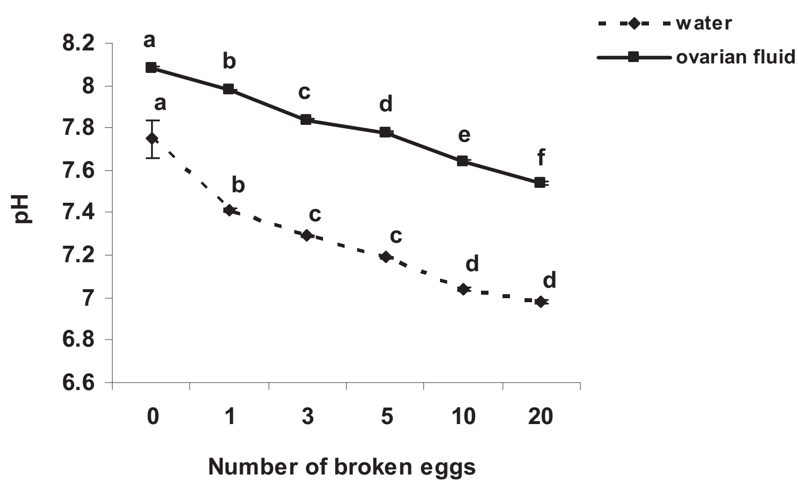

Figure 1. The changes of pH in fertilization water and ovarian fluid of Caspian brown trout in relation to the number of broken eggs. Means with same superscripts are not significantly different ($P > 0.05$)

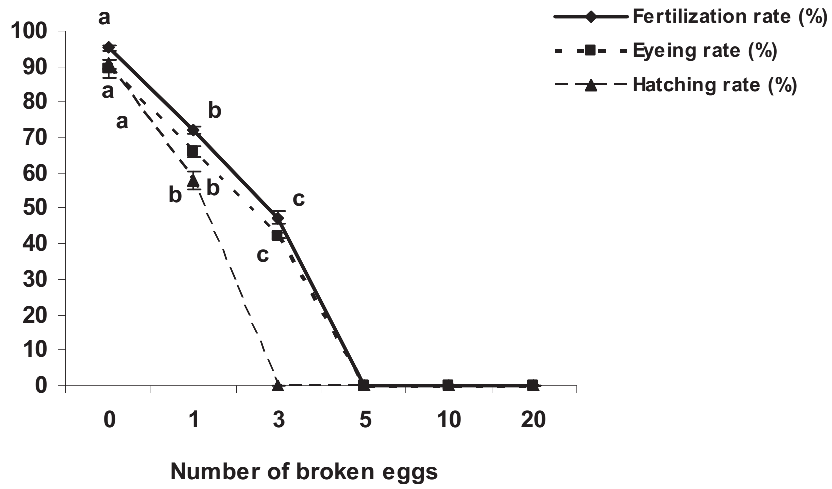

Figure 2. The changes of fertilization, eyeing and hatching rates of Caspian brown trout eggs in relation to the number of broken eggs. Means with same superscripts are not significantly different (P> 0.05)

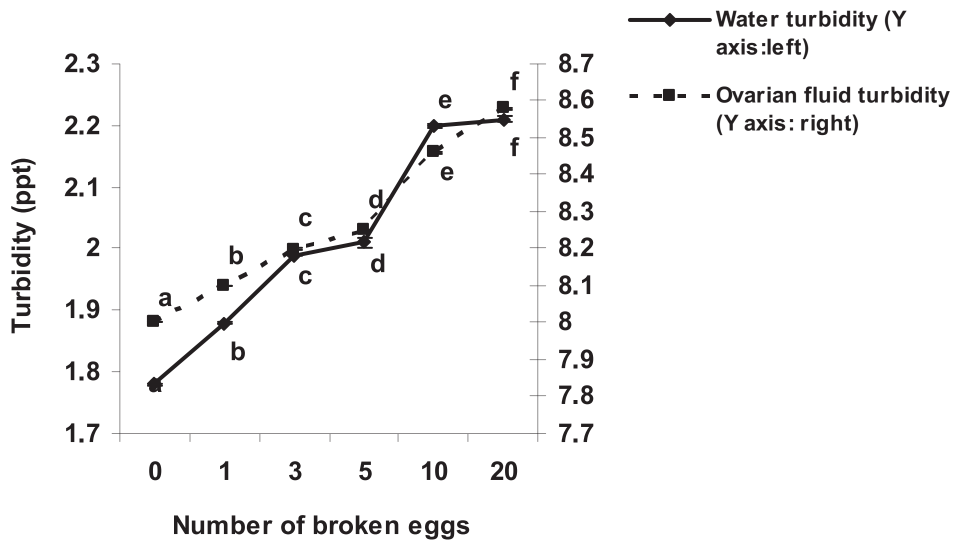

Figure 3. The changes of turbidity in fertilization water and ovarian fluid of Caspian brown trout in relation to the number of broken eggs. Means with same superscripts are not significantly different (P> 0.05)